CHARACTERIZATION OF THE HIGHLY DYNAMIC INTERFACE IN THE PLASTOCYANIN-CYTOCHROME *f* COMPLEX BY SITE-SPECIFIC 2D IR

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Protein conformational heterogeneity and dynamics, in particular those of protein side-chains, are of importance to protein function. Experimentally characterizing the contribution of these motions to function is complicated due to the diverse timescales and spatial heterogeneity inherent to proteins. Two-dimensional infrared (2D IR) spectroscopy has emerged as a powerful tool for the direct measurement of dynamics and conformational heterogeneity due to its high spatial and temporal resolution. This technique can be applied to the study of fast, protein side-chain motions by site-specifically incorporating unnatural amino acids with frequency-resolved absorptions in the "transparent frequency" region (1800 - 2500 cm^{-1}). For example, cyanophenylalanine (*CN*Phe) can be introduced in a protein in place of a native Tyr or Phe with minimal perturbation to the native protein structure. In this study, *CN*Phe was introduced in three distinct locations on the binding surface of the protein, plastocyanin (Pc) to investigate how the local environments of the chosen sites were impacted by the binding of its electron transfer partner, cytochrome *f* (cyt *f*). The data suggests the Pc-cyt *f* complex has a highly mobile interface, supporting the model of a highly populated encounter complex. This study highlights the potential of 2D IR spectroscopy to reveal new biological insights of dynamic protein complexes and further demonstrates 2D IR spectroscopy as a routine measurement of protein dynamics.